

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of: Lundqvist et al.

Confirmation No: 2788

Serial No.: 10/588,449

Group Art Unit: 1781

Filed: August 3, 2006

Examiner: Hamid Badr

For: Preparation of Dough-Based Product

DECLARATION UNDER 37 CFR 1.132

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

I, Tina Nørgaard-Salomonsen, do hereby state and declare that

1. In 2005 I obtained a Master of Science (MSc) in Food Science at The Royal Veterinary and Agricultural University, Denmark (today part of University of Copenhagen). From 2005 until 2006, before initiating my Ph.D. work, I worked as a Research Assistant at the Department of Food Science, The Royal Veterinary and Agricultural University, Denmark. In 2009, I received a Doctorate (Ph.D.) in Food Science from University of Copenhagen, Denmark. The Ph.D. work was carried out as an industrial Ph.D. in collaboration with Danisco A/S. Since May 2009, I have been employed as a Research Scientist with Novozymes A/S, the assignee of the present application. My primary responsibilities are to develop new enzyme applications for the baking industry.

2. I have read and am familiar with the present patent application.

3. The following experiments were carried out under my direction and supervision to compare the performance of a *Bacillus halodurans* C-125 xylanase of glycoside hydrolase family 11 and having the amino acid sequence of SEQ ID NO: 2 disclosed in the above-identified application (EXP00760) and a *Bacillus halodurans* C-125 xylanase of glycoside hydrolase family 8 and having the amino acid sequence of SEQ ID NO: 8 disclosed in WO 2004/023879 (EXP02385) in baking with respect to dough properties (dough stickiness,

softness, extensibility and elasticity), and with respect to volume and crumb firmness of the baked product.

4. Bread was prepared according to the sponge-and-dough procedure as follows. First, a sponge was prepared by mixing 32.7 parts of water, 59.4 parts of wheat flour, 2.5 parts of soy oil, 5.0 parts of compressed yeast and 0.4 part of SSL (sodium stearoyl-2-lactylate). The ingredients were mixed for 1 minute at low mixing speed (90 rpm) and 4 minutes at high mixing speed (150 rpm). The sponge was fermented for 3 hours at 27°C and 86% relative humidity. Subsequently, a dough was prepared by mixing the sponge with 52.8 parts of wheat flour, 24 parts of water, 15.9 parts of high fructose syrup, 5.0 parts of compressed yeast, 2.6 parts of salt, 0.04 part of monoglycerides, 0.3 part of calcium propionate, 0.006 part of ascorbic acid and 0.003 part of ADA (azodicarbonamide). Enzymes were added according to the tables below. The ingredients were mixed with the sponge for 1 minute at low mixing speed (90 rpm) and 12 minutes at high mixing speed (150 rpm). After 10 minutes resting, the dough was scaled into smaller pieces of 435 g and again left to rest for 10 minutes. After sheeting and molding, the dough was placed in a pan and fermented for 55 min at 42°C and 86% relative humidity and afterwards baked for in an oven for 17 minutes at 225°C.

Enzymes

Enzyme	Sample ID	Activity/Concentration
Novamyl 10,000 BG	FAE-2011-0006	9810 MANU/g
EXP00760	U23U5	0.24 mg EP/ml
EXP02585	U66X4	1.65 mg EP/ml

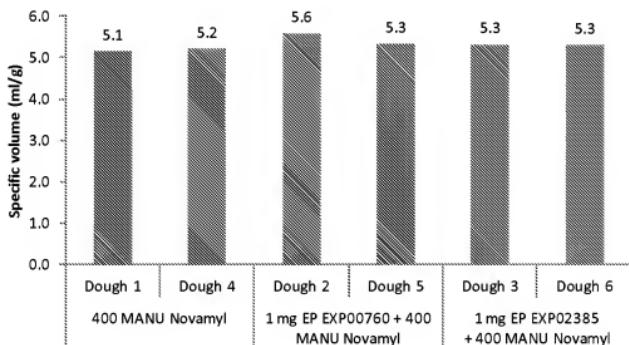
Baking trial

Enzyme	Dough #	1	2	3	4	5	6
Novamyl 10,000 BG (MANU/kg flour)	400	400	400	400	400	400	400
EXP00760 (mg EP/ kg flour)		1.0			1.0		
EXP02585 (mg EP/ kg flour)				1.0			1.0

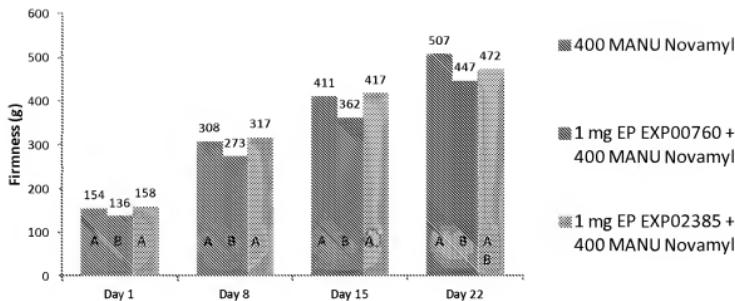
5. Dough stickiness, softness, extensibility and elasticity after mixing but before resting were evaluated by the baker using a 1-10 scale. The dough property scores are shown in the table below. The doughs supplemented with either *Bacillus halodurans* C-125 xylanase were evaluated to be slightly less sticky and elastic than the doughs containing only Novamyl. The dough properties obtained with the *Bacillus halodurans* C-125 xylanases was statistically the same.

Dough evaluation after mixing	400 MANU Novamyl		1 mg EP EXP00760 + 400 MANU Novamyl		1 mg EP EXP02585 + 400 MANU Novamyl	
	Dough 1	Dough 4	Dough 2	Dough 5	Dough 3	Dough 6
Stickiness	5	5	4	4	4	4
Softness	5	5	5	5	5	5
Extensibility	5	5	5	5	5	5
Elasticity	5	5	4	4	4	4

6. The specific volume of the bread loafs is shown in the figure below. Statistical analysis of the duplicates (multiple comparisons by Tukey-Kramer HSD procedure in SAS JMP at significance level $p < 0.05$) showed that there was no statistical difference between the three treatments with respect to volume increase.



7. Crumb firmness was evaluated 1, 8, 15 and 22 days after baking using a Texture Analyzer and a modified version of the AACC method 74-09 (RD-432-SP-1298). Three bread slices from two breads prepared from each of the six doughs were evaluated on each measurement day. Consequently, for each of the three enzyme treatments 12 bread slices were evaluated on each day (2 dough preparations x 2 breads x 3 bread slices). The averages of the firmness and elasticity measurements are shown in the figure below. Within each day, the averages were compared using the Tukey-Kramer HSD procedure (multiple comparisons) in JMP (SAS Institute) at significance level $p < 0.05$. Thus, within a given day samples not connected with the same letter are statistically different.



The results at days 1, 8, 15, and 22 demonstrate that the use of EXP00760 (the family 11 xylanase from *Bacillus halodurans* C-125) and Novamyl resulted in a softer crumb compared to only adding Novamyl. In contrast, the use of EXP02385 (the family 8 xylanase from *Bacillus halodurans* C-125) and Novamyl did not have an effect on the firmness of the bread (i.e., there was no statistical difference when compared to the samples containing only Novamyl).

These results demonstrate that the family 11 xylanase from *Bacillus halodurans* C-125 (SEQ ID NO: 2 of the present application) has a significantly better effect on the firmness of bread than the family 8 xylanase from *Bacillus halodurans* C-125 (SEQ ID NO: 8 disclosed in WO 2004/023879). These results are not predicted by the prior art, and therefore are surprising and unexpected.

8. I further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further, that the statements are made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Signed this ____ day

of March 2012

Tina Nørgaard-Salomonsen